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ORIGINAL ARTICLE

Microbiological quality of non-sterile pharmaceutical products



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Abstract In microbiological terms, pharmaceutical products can be divided into two groups: sterile and non-sterile. Non-sterile drugs must satisfy the appropriate microbiological purity criteria which are included in pharmacopoeial monographs. Pharmacopoeial studies are prepared specifically with a view to ensuring that the medicinal product is therapeutically effective and safe for the patient. The analysis comprised the results of microbiological purity tests performed before the products are marketed. Total of 1285 samples of non-sterile drugs manufactured by different pharmaceutical plants in Polish were taken into study. The microbiological quality of drugs was assessed in accordance with the criteria included in the European Pharmacopoeia (EP). An analysis of test results demonstrated that the percentage of non-compliant samples was 1.87%. The groups of drugs, which the most often did not satisfy EPs' requirements, were drugs containing raw materials of natural origin (5.7%). The samples of studied drugs that did not meet the criteria contained in EP, exceed the maximum allowable microbiological count limits and contained microbes whose presence is prohibited. The most common non-compliance was the excessive levels of the maximum acceptable fungal count ($n = 12$) and the excessive the maximum acceptable aerobic microbial count ($n = 10$).

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1. Introduction

The holder of a manufacturing authorization must drugs so as to ensure that they are fit for their intended use, comply with the requirements of the Marketing Authorization and do not place patients at risk due to inadequate safety, quality or efficacy. To achieve the quality objective, it is necessary to control all stages of drugs, which covers all matters, which individually or collectively influence the quality of a product, including raw materials, the manufacturing process and the evaluation of finished product. One of control stages is the assessment of microbiological quality of medicinal products (Tyski, 2011; Guide to good manufacturing practice for medicinal products, 2013).

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Consecutive editions of the European Pharmacopoeia (EP) are useful tools in the quality assessment, as they set out the microbiological specifications, criteria and the methods for microbial examination of non-sterile products.

The methods used and results obtained should comply with the specifications and criteria outlined in the appropriate pharmacopoeia. Testing, which is performed on both raw materials and finished products, involves microbial enumeration tests for total aerobic microbial counts (TAMC) and total yeast and mold counts (TYMC), in addition to tests for the following specified micro-organisms: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella* spp., *Candida albicans*.

Microbiological assessment of non-sterile products is particularly pertinent in view of the fact that microbial contamination can reduce or even eliminate the therapeutic effect of drugs or cause drug-induced infections. Microbes presented in drugs not only makes them hazardous from the infectious standpoint, in addition may change the chemical, physical and organoleptic properties of the drugs or change the contents of active ingredients. Furthermore, microorganisms can convert drugs to toxic products.

The presence of even a low level of pathogenic microorganisms, higher levels of opportunistic pathogens or bacterial toxic metabolites, which persist even after the death of the primary contaminants can result the product ineffective.

Not only the presence of microorganisms, which cause undesirable bacterial infections is harmful, but also the presence of metabolites/toxins may cause bad symptoms even if they are included in small amounts. Some of these toxin – related diseases include diarrhea, acute gastroenteritis or abdominal pain. Symptoms vary from mild distress to stomach death, depending on the individual sensitivity to toxin, amount of ingested toxin and victim general health. Severe infections in immunocompromised people have been assigned to *Klebsiella* spp., and *Bacillus* spp. Several hospitals acquired and some outpatient acquired infections, particularly pneumonia, are also assigned to *Klebsiella* spp. (Mugoyela and Mwambete, 2010).

Reports of infections triggered by drug contamination of microbial origin led to the establishment, in the second half of the 20th century, of a special committee at the International Pharmaceutical Federation (FIP) which was tasked with drawing up guidelines regulating drug production. The works culminated in the development of Good Manufacturing Practice (GMP) guidelines. They are not a static concept but rather a dynamically developing system which allows further improvement of the production process. The GMP principles were introduced to ensure top-quality pharmaceutical products and safeguard patients' life and health.

Moreover, microbiological purity criteria were established and the requirement for final microbiological control was introduced. Also, a set of rules was postulated to regulate the question of maintaining environmental hygiene, preventing potential contaminants from gaining entry to manufacturing sites and ensuring proper storage conditions for raw materials used in production processes (Regulation of the Minister of Health, 2008, 2009). In view of the observed rapid growth of the pharmaceutical sector, rules of conduct were prescribed for the manufacturing process to ensure that appropriate quality of finished products is maintained. The guidelines are compiled in the form of Good Manufacturing Practice code. Under the Act on Pharmaceutical Law issued on 6 September 2001, GPM refers to practices "ensuring that the medicinal products

are manufactured and controlled adequately to their intended use and in compliance with the requirements included in their specifications and documents constituting a basis to issue a permit for marketing authorization of medicinal product" (Act on Pharmaceutical Law, 2001).

The aim of study was to analyze the results obtained from microbiological purity tests of non-sterile drugs by different pharmaceutical sides based in the province of Poland. The aim of study was to present types of inconsistencies profile occurring in the studied groups of drugs. The obtained results which are presented below can improve the production quality in pharmaceutical plants, inform/aware about the necessity of microbiological control production process of each drugs series and thereby improve the safety and quality of medicines.

2. Material and methods

The analysis comprised the results of microbiological purity tests performed before the products are marketed. Total of 1285 samples of non-sterile drugs in Polish and manufactured by different pharmaceutical plants were taken into study. This was a cross-sectional study conducted at Department of Genetics and Pharmaceutical Microbiology between 2011 and 2013.

The following microbial assays were performed: total aerobic microbial count (TAMC), total mold and yeast count (TYMC), presence and count of bile tolerant Gram-negative bacteria (*Enterobacteriaceae*), presence of *P. aeruginosa*, *E. coli* and *C. albicans* in 1 g/mL product and *Salmonella* spp. in 10 g/mL. The microbiological quality of drugs was assessed in accordance with the criteria laid down in the European Pharmacopoeia (EP) (Table 1).

The methods and media described in the EP were used. The viable aerobic mesophile bacteria count was performed by plating 1 ml of decimal dilutions on casein soy agar. Plates were incubated at 30 °C for 5 days. The results are displayed as colony forming units per gram of sample (CFU/g). The molds and yeast count was performed by plating 1 ml of decimal dilutions on Sabouraud dextrose agar. Plates were incubated at 25 °C for 5–7 days. For qualitative examination, 10 mL of prepared sample was added to 100 mL of casein soya bean digest broth. The further procedure was dependent on the determination of the absence or limited occurrence of specified microorganism that may be detected:

- *S. aureus* – incubation (24 h, 35 °C) and transmission on Mannitol Salt Agar.
- *P. aeruginosa* – incubation (24 h, 35 °C) and on Cetrimide Agar.
- *E. coli* – incubation (24 h, 35 °C) and transmission to MacConkey broth (incubation 48 h, 44 °C), then transmission on MacConkey agar.
- *Salmonella* spp. – incubation (24 h, 35 °C) and transmission to Rappaport Vassiliadis (incubation 24 h, 35 °C) then transmission on Xylose, lysine, deoxycholate agar.
- *Enterobacteriaceae* – incubation (2–5 h, 20–25 °C), transmission to enterobacteria enrichment broth- Mossel (incubation 24–48 h 35 °C), then transmission on violet red bile glucose agar.

Table 1 Acceptance criteria for microbiological quality of non-sterile dosage forms (European Pharmacopoeia (2010).

Route of administration	TAMC (CFU/g)	TYMC (CFU/g)	Specified micro-organisms
Non-aqueous preparations for oral use	10 ³	10 ²	Absence of <i>Escherichia coli</i> (1 g)
Aqueous preparations for oral use	10 ²	10 ¹	Absence of <i>Escherichia coli</i> (1 g)
Cutaneous use	10 ²	10 ¹	Absence of <i>Staphylococcus aureus</i> (1 g) Absence of <i>Pseudomonas aeruginosa</i> (1 g)
Vaginal use	10 ²	10 ¹	Absence of <i>Staphylococcus aureus</i> (1 g) Absence of <i>Pseudomonas aeruginosa</i> (1 g) Absence of <i>Candida albicans</i> (1 g)
Oral dosage forms containing raw materials of natural origin, for which antimicrobial pretreatment is not feasible	10 ⁴	10 ²	Absence of <i>Escherichia coli</i> (1 g) Absence of <i>Staphylococcus aureus</i> (1 g) Absence of <i>Salmonella</i> (10 g) Not more than 10 ² <i>Enterobacteriaceae</i> and certain other gram-negative bacteria (1 g)
Herbal medicinal products to which boiling water is added before use	10 ⁷	10 ⁵	Not more than 10 ³ <i>Escherichia coli</i> (1 g) Absence of <i>Salmonella</i> (1 g)

Further identification was carried out using automatic Vitek 2 system Compact (bioMérieux). On each assay, the air in the laminar flow cabinet was controlled during the test development. Also checked were the sterility of the used media and the lack of inhibitory power of the sample as described in the EP (European Pharmacopoeia, 2010).

3. Results

A total of 1285 samples of non-sterile pharmaceutical drugs in the form of tablets, capsules, ointments and the syrup with various routes of administration and compositions were tested. The dominant sub-group among the drugs under study was orally administered non-aqueous drugs and they represented 55.5% ($n = 714$) of tested samples, while aqueous preparations for oral use constituted 8.1% ($n = 104$). Drugs containing raw materials of natural (plant-based) origin including: oral dosage forms, for which antimicrobial pre-treatment is not feasible accounted for 17.7% ($n = 227$) and herbal medicinal products formulated with plant-based substances treated with boiling water before use were 3.1% ($n = 40$). The percentages determined for other study drugs were respectively: 8.6% ($n = 110$) for cutaneous use and 7.0% ($n = 90$) for vaginal use. The smallest percentage participation of the study drugs (3.1% ($n = 40$)) was herbal medicinal products to which boiling water is added before use. Detailed analysis of the obtained results, the type of non-compliance and their prevalence are presented in Table 2.

An analysis of test results showed the percentage of non-compliant samples to be low (1.87%). Study drugs were non-compliant with the EP criteria due to: excessive microbial counts and the presence of pathogens prohibited by the EP. The most common non-compliance was the excessive levels of the maximum acceptable fungal count ($n = 12$) and then, the excessive the maximum acceptable aerobic microbial count ($n = 10$).

The groups of drugs, which the most often did not satisfy requirements of EP were drugs containing raw materials of natural origin (5.7%). In four samples, the limit of aerobic microorganisms was exceeded (the highest noted bacteria number was 4.3×10^5 CFU/g), while in seven samples the limit of molds was exceeded (the highest contamination was

1.4×10^5 CFU/g). Among this group of drugs, in three samples also exceeded the limit of *Enterobacteriaceae* bacteria family was observed. There were: *Pantonea* spp., *Raoutella planticola*, *Citrobacter braakii*. The presence of *E. coli* in two samples was detected.

In addition, in two tested samples at the same time more than one non-compliance were recorded (first sample: exceeded count of aerobic bacteria, molds and bacteria from family *Enterobacteriaceae*, second sample: exceeded count of aerobic bacteria and *Enterobacteriaceae* bacteria).

Seven contaminated samples (0.98%) in group of non-aqueous preparations for oral use were noted. In three samples, exceeded limit of aerobic microorganisms was observed (the highest noted bacteria number was 1.6×10^5 CFU/g), while the highest molds contamination in four samples was 3.4×10^3 CFU/g. Three samples, in herbal medicinal products to which boiling water is added before use, did not comply with the EP requirements. In two samples aerobic bacteria were presented, in one molds were detected. In oral drugs containing water only one contaminant was observed.

Aerobic bacteria, which were contaminations of studied drugs belonged to genus: *Bacillus*, *Micrococcus*, *Enterococcus*, while identified molds: *Aspergillus*, *Rhizopus*, *Alternaria*, *Mucor*. Bacteria: *Staphylococcus aureus*, *P. aeruginosa*, *Salmonella* spp., and yeast *C. albicans* were not detected in any of tested drugs groups.

4. Discussion

Drugs which do not require sterility regardless of their dosage form and route of administration must conform to the microbiological purity criteria set out in an appropriate edition of the EP. Control of medicinal products is a preventative mechanism which aims to prevent the launch of harmful products on the consumer market. Many pathogens or, more specifically, metabolites which they produce, have a capacity to either break down or inactivate the drug substance. Furthermore, drugs are taken by people with compromised immunity, so in order to prevent drug-induced infections consecutive editions of the Pharmacopoeia impose limits on microbial contamination.

In general terms, drug-induced infections occur only sporadically; however, they can also take the form of hospital

Table 2 Causes, types and prevalence of non-compliances.

Number of samples incompatible with EP requirements	Causes of non-compliances							
	Exceeded the limit of aerobic microorganisms		Exceeded the amount of fungi		Exceeded the number of Gram-negative <i>Enterobacteriaceae</i> bacteria CFU/g		Presence of <i>E. coli</i>	
	Number of samples	The level of bacteria CFU/g	Number of samples	The level of fungi CFU/g	Number of samples	The level of Gram(–) CFU/g	Number of samples	Presence of <i>E. coli</i>
Non-aqueous preparations for oral use $n = 7$	$n = 3$	1.6×10^5 6.6×10^3 8.2×10^3	$n = 4$	6.0×10^2 5.6×10^2 8.0×10^2 3.4×10^3	–	–	–	–
Aqueous preparations for oral use $n = 1$	$n = 1$	4.2×10^4	–	–	–	–	–	–
Cutaneous use $n = 0$	–	–	–	–	–	–	–	–
Vaginal use $n = 0$	–	–	–	–	–	–	–	–
Oral dosage forms containing raw materials of natural origin, for which antimicrobial pretreatment is not feasible $n = 13^a$	$n = 4$	6.4×10^4 3.2×10^5 4.3×10^5 8.4×10^4	$n = 7$	4.3×10^3 1.4×10^4 6.4×10^2 2.1×10^3 3.4×10^3 1.4×10^5 8.0×10^2	$n = 3$	$> 1.0 \times 10^3$ $> 1.0 \times 10^3$ $> 1.0 \times 10^4$	$n = 2$	<i>E. coli</i> <i>E. coli</i>
Herbal medicinal products to which boiling water is added before use $n = 3$	$n = 2$	6.1×10^7 8.5×10^7	$n = 1$	3.2×10^5	–	–	–	–

^a In two samples more than one non-compliance were reported.

acquired infections (HAI) of epidemic nature. Several different cases of infections caused by the use of contaminated medications have been reported in the scientific literature. The first case of a drug-induced infection was reported in 1907, when the bubonic plague vaccine was found to be contaminated with tetanus bacilli. Another documented case was e.g.: *Salmonella* infections, caused by tyrocidine tablets, and by pancreatic powder; *Pseudomonas cepacia* present in iodated povidone; ocular infections, caused by *P. aeruginosa* in hydrocortisone ointment and other (Kallings et al., 1966; Glencross, 1972; Berkelman et al., 1984).

Since today drugs are manufactured on an industrial scale, the focus of control has been shifted toward the assessment of the manufacturing site and the production process. Drug manufacturers are responsible for producing medicinal products of appropriate quality. The system of drug control consists of three stages: (a) drug registration control, (b) production control (i.e. raw materials, manufacturing process, finished product) and (c) distribution control. The three stages combine for the ultimate goal of preventing defective products from reaching the patient. In this study we present the results of microbiological purity control of drugs at the stage of final product – prior to release.

There have been many international reports on the irregularities in the process of drug production resulting in poor quality products being granted marketing authorizations. Długaszewska et al. reported that over a 10-year period of analysis the mean percentage of non-compliant samples was 0.7%. An analysis of the results of microbiological purity assays of compounded drugs showed that as much as 5.6% of them failed to comply with applicable standards. The major non-compliance was excessive bacterial count, followed by fungal and Gram-negative bacterial count (Długaszewska et al., 2008). Charnock, in his study, evaluated microbial content of seventy-seven registered trademark non-sterile pharmaceuticals products distributed in Norway. All the products examined complied with current regulations with respect to the numbers and types of microbes isolated, indicating the effectiveness of existing production practices in meeting existing standards. Gram-positive endospore-forming rods accounted for the majority of the bacteria isolated. Gram-negative rods for the most part in incidental numbers were presented. However, some of these were of species that have been previously indicated as opportunistic pathogens and which should be considered as objectionable in pharmaceuticals (Charnock, 2004).

Among our studied 1285 drugs samples, 24 samples showed unconformities with EP requirements. The most common cause of non-compliance was the excessive levels of the maximum acceptable fungal count. Isolated molds belonged to genus: *Aspergillus*, *Rhizopus*, *Alternaria*, *Mucor*, yeast did not be detected. Molds can produce mycotoxins which can be carcinogenic and mutagenic. They can also cause acute and chronic poisoning, allergies, diseases of the respiratory and digestive systems, and liver damage (Wu et al., 2014).

In our study, three samples contained Gram-negative *Enterobacteriaceae* bacteria, while two samples were contaminated with *E. coli*. The samples in which Gram-negative bacterial con-

tamination limits were exceeded and *E. coli* was detected were oral dosage forms containing raw materials of natural origin, for which antimicrobial pre-treatment is not feasible. The source of contaminants may were in the natural environment (water, soil). Crops may also become indirectly contaminated through poorly composted organic fertilizers. Ruminant feces may be a source of contamination with *E. coli* bacteria which form a part of their natural intestinal flora. Observed contaminants of bacteria from *Enterococcus* genus can be associated with raw plants or they may also indicate contamination with fecal material because these bacteria are inhabitants of the gastrointestinal tract of humans and other animals.

The results of our study demonstrated that the percentage of EP-non-compliant samples before market was 1.87%, which leads to conclude that: (1) The drugs microbiological control in accordance with GMP and EP is required at each stage of production, particularly at the stage of the final product prior to release (2) must be subjected to control each series of produced drugs. Quality control of pharmaceutical products is not only important for compliance with standards, but also reduces risk to the end user, and, consequently, to the manufacturer.

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